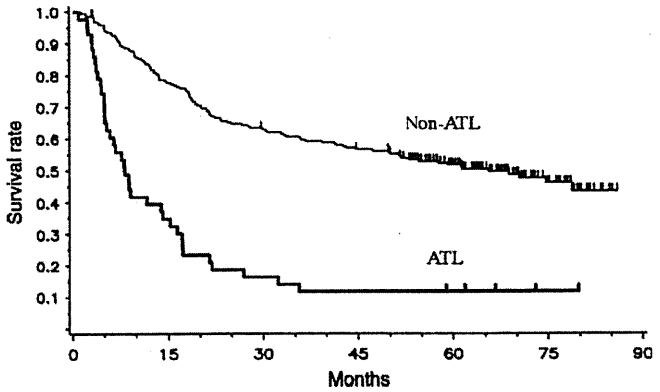

Figure 2. The Kaplan–Meier estimate of overall survival for all patients treated with LSG4 in relation to disease entity. ATL, adult T-cell leukemia–lymphoma.

JCOG9002, a randomized Phase III study, evaluated the dose-intensification strategy for doxorubicin and cyclophosphamide in the third-generation multiagent combination chemotherapy, LSG9 (VEPA-B/FEPP-AB/M-FEPA every 10 weeks; three courses, 28 weeks in total), when compared with second-generation combination chemotherapy, modified LSG4 (mLSG4) (VEPA-B/FEPP-B/M-FEPA every 14 weeks; four courses, 54 weeks in total) (14). Planned dose intensity (DI)/week of DOX and CPA was 1.9- and 1.5-fold higher in LSG9 than in mLSG4, respectively. Median actual DIs of DOX and CPA were 1.6- and 1.2-fold higher in LSG9 than in mLSG4, respectively, with no difference in 5-year OS and the %CR, revealing no survival benefit of the DI strategy.

In 1993, an intergroup US Phase III study revealed that CHOP remained the standard therapy for aggressive NHL when compared with second- and third-generation regimens (15). Also, the international prognostic index (IPI) for patients with aggressive NHL was developed (16). Based on these findings, JCOG-LSG changed the treatment strategy for aggressive NHL from the multiagent chemotherapies to the dose intensification of key agents, and initiated several Phase II studies of regimens based on CHOP for patients divided by IPI risk grouping. Among them, JCOG9508, a Phase II study of standard CHOP every 3 weeks for low and low-intermediate (L/LI)-risk patients with advanced aggressive NHL, revealed that the full dose of CHOP was feasible and effective for Japanese patients as for westerners (17).

JCOG9505, a randomized Phase II study of CHOP every 2 weeks (CHOP-14) and dose-escalated CHOP both supported with the prophylactic use of G-CSF in high-intermediate and high (HI/H)-risk aggressive NHL, revealed that the former was more promising with similar %CR and progression-free survival (PFS) rates, but lower toxicity (18). Following the results of JCOG9505, a randomized Phase III (JCOG9809) study comparing CHOP-14 with CHOP-21 in patients newly diagnosed with advanced-stage aggressive NHL at all IPI risk was conducted (19,20). A planned interim analysis revealed that dose intensification with interval shortening of CHOP did not prolong PFS in advanced, aggressive NHL, resulting in an early stop to the study (19), and long-term follow-up confirmed the results (20). There were no remarkable differences in PFS or OS between the two arms. Secondary malignancies, including myelodysplastic syndrome, were significantly more frequent in the CHOP-14 arm.

Since around 2000, rituximab (R), an anti-CD20 monoclonal antibody, has changed the treatment strategy for all CD20-expressing B-cell neoplasms including diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL) and chronic lymphocytic leukemia (21). Six to eight courses of rituximab plus CHOP (R-CHOP) every 3 weeks (R-CHOP-21) is now the standard therapy for DLBCL of all risk groups (22). To evaluate the efficacy of DI of rituximab, a randomized Phase II/III study, JCOG0601, is now ongoing comparing the standard with weekly R-CHOP for DLBCL, based on the results of a pharmacokinetic analysis of rituximab monotherapy in a Phase II study for relapsed or refractory aggressive B-NHL (23).

High-dose chemotherapy (HDC) supported with auto-HSCT has been evaluated after induction chemotherapy to improve the prognosis for poor-risk aggressive NHL. However, the results have been controversial in the era before rituximab (24). The US intergroup has conducted a randomized Phase III study evaluating the efficacy of the addition of HDC/auto-SCT after R-CHOP for high-risk DLBCL (25). Considering the next step in the clinical trial for poor-risk DLBCL, JCOG-LSG is now conducting a randomized Phase II study of R-CHOP every 2 weeks (R-CHOP-14) versus R-CHOP-14 followed by CHASER (cyclophosphamide, cytarabine, dexamethasone, etoposide, rituximab) as induction therapy prior to HDC, LEED (melphalan, cyclophosphamide, etoposide, dexamethasone) and auto-HSCT in poor-risk DLBCL (JCOG0908).

Adult T-cell Leukemia–Lymphoma

JCOG-LSG has consecutively studied the treatment of ATL, which was discovered as a new disease entity just before the establishment of LSG. The earlier trials revealed a poor prognosis of ATL when compared with the other aggressive NHL (JCOG7801, 8101, 8701) (11,13,26). Then, a nationwide survey in 854 patients with ATL in Japan revealed that the major prognostic factors were advanced PS, high lactic dehydrogenase (LDH) level, age of 40 years or more, more than three involved lesions and hypercalcemia by multivariate analysis (27). Also, a classification of clinical subtypes into acute, lymphoma, chronic and smoldering types was proposed based on prognostic factors and clinical features of the disease (28). This subtype classification has been reported to be reproducible for predicting prognosis and has been widely applied for treatment decisions. Recently, a treatment strategy based on the clinical subtype classification and prognostic factors was suggested, including a watchful waiting approach, chemotherapy, antiviral therapy,

allo-HSCT and targeted therapies for clinical trials and clinical practice (29).

The disappointing results with conventional chemotherapies in the 1980s and the proposal for a subtype classification of ATL have led to a search for new active agents focusing on aggressive ATL in JCOG-LSG. The first Phase II study of combination chemotherapy with pentostatin (2′-deoxycoformycin, an inhibitor of adenosine deaminase) was conducted exclusively against aggressive ATL, based on the promising results of pentostatin monotherapy for relapsed or refractory ATL patients (30). However, the results were disappointing with a median survival time (MST) of 7 months similar to previous studies by JCOG-LSG (31). The next Phase II trial (JCOG9303) consisting of vincristine, cyclophosphamide, doxorubicin and prednisone (VCAP); doxorubicin, ranimustine and prednisone (AMP); and vindesine, etoposide, carboplatin and prednisone (VECP) intensified with the prophylactic use of G-CSF revealed a promising response rate and MST superior to those obtained by our previous trials, despite considerable hematological toxicity (32). Based on the promising results of JCOG9303, we conducted a Phase III trial comparing modified (m)LSG15 (VCAP–AMP–VECP) with CHOP-14 both supported with G-CSF and intrathecal prophylaxis. The longer survival at 3 years and higher %CR with VCAP–AMP–VECP compared with CHOP-14 suggest that the former is a more effective regimen at the expense of greater toxicity, providing the basis for future investigations in the treatment of ATL (33). However, the MST of 13 months still compares unfavorably to other hematologic malignancies.

Allo-HSCT is now recommended for the treatment of young patients with aggressive ATL (29). To evaluate the promising efficacy of allo-HSCT, possibly associated with a graft-versus-ATL effect, especially in view of a comparison with intensive chemotherapy, a prospective multicenter Phase II study of mLSG15 chemotherapy followed by allo-HSCT, comparing the results with historical control in JCOG9801, has been initiated as JCOG0907.

A combination of interferon-α (IFN) and zidovudine (AZT) was reported as promising for the treatment of ATL in small Phase II trials in 1995 from the USA and Europe (34–36). Recently, in a worldwide retrospective analysis, it was reported that this combination might be effective especially for indolent ATL when compared with watchful waiting (37). A prospective Phase III study evaluating the efficacy of IFN/AZT when compared with watchful waiting for indolent ATL is to be initiated (JCOG PC908) under the highly advanced medical technology assessment system because IFN and AZT are not covered for ATL by the National Health Insurance in Japan.

Lymphoblastic Lymphoma/Acute Lymphoblastic Leukemia

Lymphoblastic lymphoma (LBL)/acute lymphoblastic leukemia (ALL) is a malignancy of immature T/B lymphoblasts and takes an acute and aggressive course affecting relatively young individuals. Treatment of child ALL/LBL has much advanced. In contrast, advances for adults have been modest.

JCOG7801 and JCOG8101 revealed that T-LBL and ATL had a poor prognosis compared with other NHLs. Then, a Phase II study of a short-term, combination chemotherapy without maintenance therapy (JCOG8702) for LBL/ALL revealed that a fraction of adult patients with the disease were curable with a short-term, six-drug chemotherapy regimen (38). The next Phase II study (JCOG9004), G-CSF-supported, intensive post-remission chemotherapy and subsequent allo/auto-SCT, revealed that survival and PFS were improved from JCOG8702 in adult ALL and LBL (39). The next chemotherapeutic regimen with the intensified induction and post-remission chemotherapy with auto/allo-HSCT in JCOG9402 was feasible; however, this study failed to show improvements in long-term follow-up results when compared with the historical control JCOG9004 (40).

To further improve the therapeutic outcomes of adults with LBL/ALL, novel strategies are warranted such as risk-adapted treatment for bcr-abl-positive poor prognostic ALL with abl inhibitors. Partly because of the relatively low incidence of LBL/ALL, JCOG-LSG never activated clinical studies after JCO9402.

Hodgkin's Lymphoma

HL is the most chemo/radio-sensitive malignancy among malignant lymphomas, and clinical trials for the disease have steadily produced standard therapies. However, trials are less frequently conducted in Japan and other Asian countries because of a low incidence. Sequential Phase II studies for advanced HL (JCOG8905 and 9305) of C-MOPP (cyclophosphamide, vincristine, procarbazine and prednisone)/ABVd (doxorubicin, bleomycin, vinblastine and dacarbazine) and ABVd, respectively, both with a dose reduction of dacarbazine (250 mg/m^2) because of severe emesis in previous studies in Japanese, confirmed a similar efficacy to those from the USA and Europe (41–43). Safety and efficacy profiles of dacarbazine included in C-MOPP/ABVd and ABVd led to the approval of dacarbazine for clinical use covered through the National Health Insurance by MHLW in Japan without industrial trials.

The next Phase II study of ABV deleting dacarbazine with increased dose of doxorubicin followed by IF-RT (JCOG9705) revealed at the interim analysis that the 2-year PFS was significantly inferior to JCOG9305 (ABVd), suggesting that dacarbazine is a key agent for the treatment of HL (44).

A recent meta-analysis of the two JCOG studies in HL revealed two independent factors for OS, male and an elevated serum LDH, after a multivariate analysis (JCOG0108A) (45). Partly because of the low incidence of HL in Japanese, JCOG-LSG never conducted clinical studies after JCO9705. Recent studies from westerners revealed the efficacy of further risk-adaptive treatment for HL, lower dose of chemo/radio-therapy for those at low risk and more

intensive chemotherapy for those at high risk (46). New agents for HL include anti-CD30 monoclonal antibodies. Furthermore, therapy adjustment after an interim analysis of the response by F-fluorodeoxyglucose positron emission tomography–computed tomography (PET–CT) is now suggested. JCOG-LSG is now planning a new trial for HL including PET/CT scans.

Multiple Myeloma

MM is a progressive and incurable malignancy of plasma cells affecting mainly aged individuals. Alkylators and steroids have been the key drugs for remission induction, but MST was around 3 years without a plateau in the survival curve (47). A Phase II study of COP (cyclophosphamide, prednisolone)–MP (melphalan, prednisolone) for untreated overt MM patients (JCOG8906) revealed a similar efficacy to those from the USA and Europe (48). A subsequent randomized Phase III study comparing modified (m)COP-MP with/without ranimustine for untreated overt MM (JCOG9301) revealed that addition of ranimustine to mCOP/MP has no benefit for survival, despite improving the response rate and PFS, similar to findings of other studies evaluating the addition of new agents to alkylators and steroids in MM (49).

Both Phase III and II studies on untreated overt MM patients who were ineligible for HDC/auto-HSCT and eligible, respectively (JCOG0112 and JCOG0005-DI), were terminated early because of poor patient accrual and the results of a planned interim analysis, respectively (50). The planned interim analysis of JCOG0005-DI, when 16 of the 50 planned patients were enrolled, revealed that the primary endpoint, response rate, was less than the lower threshold associated with violation in two patients who underwent allo-HSCT instead of scheduled auto-SCT because of availability of HLA-matched sibling donors. Since 2000, several promising new agents have been incorporated in standard therapy for the disease (51). Following the results, LSG is now conducting a randomized Phase II study of bortezomib, a proteasome inhibitor, plus dexamethasone versus thalidomide, an immune modulator, plus dexamethasone for relapsed or refractory MM (JCOG0904).

Indolent B-cell NHL

Advanced FL and other low-grade B-cell lymphomas are clinically indolent but non-curable diseases in most patients. The prognosis for lymphomas has been improved by adding rituximab to chemotherapy (21). However, the optimal combination schedule of chemotherapy and rituximab has not been elucidated. We attempted to determine whether patients with indolent B-cell NHL would have long-term benefits from G-CSF-supported, dose-dense immune-chemotherapy which potentiates the antibody-dependent cell-mediated cytotoxicity of rituximab by comparing R-CHOP-21 versus R-CHOP-14 (JCOG0203) (52). However, the dose-dense strategy failed to improve PFS at the median follow-up time of 5.2 years. We are now planning to follow-up the patients enrolled in this study to further evaluate the long-term prognosis of this indolent disease and potential late complications including secondary malignancies.

Mantle Cell Lymphoma

Mantle cell lymphoma (MCL) is a progressive, non-curable and relatively rare B-cell lymphoma derived from mantle zone B-cell with BCL1 translocation. In contrast to DLBCL and FL, addition of rituximab to CHOP did not improve the survival of MCL and HDC/ASCT has been reported as promising (53). Therefore, LSG is now conducting a single-arm Phase II study of R-high-CHOP followed by CHASER and HDC, LEED and auto-HSCT for previously untreated advanced-stage MCL (JCOG0406).

Localized Nasal NK/T-cell Lymphoma

Localized nasal NK/T-cell lymphoma is a refractory lymphoma relatively frequent in East Asia. Both the international project on PTCL and JCOG meta-analysis on T-NHL (JCOG0902A) revealed that the diagnosis of NK/T-cell lymphoma was poor (54,55). A Phase I/II study (JCOG0211-DI) of concurrent radiotherapy (50 Gy) and three courses of dexamethasone, etoposide, ifosfamide and carboplatin (DeVIC) consisting of multidrug resistance-non-related agents revealed that 2/3 dose of DeVIC and radiation was a safe and effective treatment when compared with a historical control of radiotherapy alone (56). A correlative study is ongoing to elucidate risk factors for relapse because PFS was not sufficient.

FUTURE ISSUES FOR JCOG-LSG

JCOG-LSG has conducted clinical trials for aggressive NHL since 1970, which has been divided into DLBCL, MCL, LBL/ALL, ATL and NK/T-NHL later, HL, MM and indolent-B-NHL, as shown in Fig. 1, to evaluate combined modality, dose intensification and incorporation of new agents in multidisciplinary treatment for lymphoid malignancies. LSG, now consisting of 47 institutions, is a relatively large group in JCOG, using much of the resources of the JCOG Data Center and Committees. JCOG-LSG initiated several studies independent from the JCOG-Data Center and supported by its own data center. However, numbers of patient enrollment in LSG have decreased in the last several years mainly due to the small number of ongoing trials. It takes longer to activate LSG protocols when compared with those by other cancer groups in JCOG mainly because of many disease entities, diverse prognosis and complex response criteria in each major disease entities, such as DLBCL, ATL and MM. Lymphoid malignancies are relatively rare; however, the spectrum is diverse consisting of 81

disease entities from indolent to aggressive in the WHO classification of 2008 (1). One way to conduct future LSG trials is to focus more on each disease entity as in the case of ATL and ENK/TML. The other is grouping the entities by treating modalities as in the case of several CD20-expressing low-grade B-cell lymphomas with rituximab-containing chemotherapy. It is desirable that clinical trials in LSG be based on disease entity, and if possible with risk grouping as in the case of trials for DLBCL and ATL. However, most of the diseases are rare and some of them take similar clinical courses including prognosis and response to therapies. On this issue, peripheral T-cell lymphomas other than ATL and T/NK ML, and low-grade B-cell lymphomas are the major two categories of disease-entity grouping.

Not only the complexity in lymphoma classification mentioned above, but also that in response criteria for lymphoma, ATL and MM which are, respectively, distinct from RECIST (response evaluation criteria in solid tumors) for other solid cancers has made the trials in LSG difficult. Recent revised criteria for lymphoma, which are applied in the JCOG0601 study, incorporating the PET/CT scan for decision of CR, might reduce the difficulty (57).

As described, JCOG-LSG has conducted clinical trials for establishing standard therapies. To keep and further upgrade the originality of JCOG-LSG trials in relation to similar cooperative study groups in the USA and Europe, several points are important including major target diseases, risk grouping for stratification and major phase of the trials. Since its establishment, JCOG-LSG has consecutively focused on diseases relatively common in Japan such as DLBCL, MM, ATL and NK/T-NHL. Recent advances in molecular-targeting therapy introduced many promising new agents for the diseases and other lymphoid malignancies. This promotes research on lymphoid malignancies for the early development of a new-standard combination therapy with the new agents in Japan. However, for the evaluation of new agent-combining treatment in Japan, JCOG-LSG should go side by side with those through industry-supported new agent trials to contribute to further improvement in the treatment of lymphoid malignancies with less lag from foreign developments. For instance, the highly advanced medical technology assessment system, which was enacted recently, would be one way of reducing the lag in Japan. Multigroup trials, including global ones and bridging studies, are another way.

Correlative studies in clinical trials have changed the next step of stratified treatments. For instance, ATL and T-LBL patients were excluded from subsequent JCOG trials for aggressive NHL since their clinical diagnosis was found to be poor in early trials (11–13). Future correlative studies in JCOG-LSG, retrospective and prospective and pathological and molecular analysis, should change the stratification of the clinical trials in future for risk-adaptive treatment. For that purpose, a banking system, which is now being established in JCOG for blood and tissue samples, is warranted. Fortunately, in the case of lymphoid malignancies, relatively easy access to samples of the neoplasm can promote correlative studies.

Lastly, as a member of cancer groups in JCOG, LSG will continue efforts to produce valuable and reliable evidence for the improvement of therapy for patients with lymphoid malignancies as rapidly as possible.

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Conflict of interest statement

None declared.

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ORIGINAL ARTICLE

Retrospective analysis of primary gastric diffuse large B cell lymphoma in the rituximab era: a multicenter study of 95 patients in Japan

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Abstract Primary gastric diffuse large B cell lymphoma (PG-DLBCL) is common subtype of extranodal non-Hodgkin lymphoma. The optimal treatment strategy for PG-DLBCL in the rituximab era still remains unknown. To evaluate clinical outcomes of PG-DLBCL in the rituximab era, we conducted a retrospective, multicenter analysis of 95 patients with PG-DLBCL. In 58 patients with localized disease, 3-year progression-free survival (PFS) and overall survival (OS) were 91% and 91% for patients with six cycles of rituximab plus CHOP (R-CHOP) and 92% and 95% for patients with three to four cycles of R-CHOP plus radiotherapy (Log-rank test, P=0.595 and P=0.278, respectively). In 37 patients with advanced disease, 3-year PFS and 3-year OS were 43% and 64% for patients with R-CHOP chemotherapy with or without radiotherapy. On multivariate analysis, advanced stage and elevated serum LDH levels were independent predictors of survival in patients with PG-DLBCL. One patient with localized disease relapsed in lymph node, and eight patients with advanced disease relapsed in lymph node (n=3), stomach (n=2), central nervous system (CNS; n=2), and duodenum (n=1). Intriguingly, CNS relapse developed within 6 months after initial series of treatment (4.9 and 5.8 months, respectively), and stomach relapse developed in later phase (27.2 and 32.9 months, respectively). Clinical outcomes of PG-DLBCL were extremely favorable for localized-stage patients in the rituximab era, although these might be poor for advanced-stage patients even in the rituximab era. Further prospective analyses are warranted.

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Keywords Primary gastric lymphoma · Diffuse large B cell lymphoma · Rituximab · Radiotherapy · Relapse

Introduction

Primary gastric diffuse large B cell lymphoma (PG-DLBCL) is the most common histologic type of extranodal non-Hodgkin lymphoma [1]. Regarding initial treatment for this condition, various modalities have long been used, including surgery, chemotherapy, and radiotherapy, either alone or in combination [2]. In a randomized controlled trial in patients with localized-stage PG-DLBCL, chemotherapy alone had a 90% cure rate, and 10-year overall survival was equivalent to that of surgery plus chemotherapy [3] while, in a subsequent prospective study in patients with localized-stage PG-DLBCL, chemotherapy followed by radiotherapy was shown to be highly effective [4]. These results lead to the replacement of surgical resection with more stomach-preserving therapy and chemotherapy followed by radiotherapy is commonly used treatment in localized disease. Nevertheless, it remains unclear whether optimal treatment is provided by chemotherapy alone or chemotherapy followed by radiotherapy [5].

With regard to advanced-stage PG-DLBCL, a prospective study by the *Groupe d'Etude des Lymphomes de l'Adult* (GELA) showed that gastrointestinal lymphomas behaved similarly to nodal lymphomas in patients treated with chemotherapy alone [6]. Since the appearance of this study, patients with advanced-stage PG-DLBCL have been mainly treated with chemotherapy alone because of the effectiveness and feasibility [1, 7].

The advent of rituximab, a chimeric anti-CD20 monoclonal antibody, has changed clinical treatment for DLBCL. A number of randomized clinical trials, conducted mainly for advanced-stage DLBCL, have shown that the addition of rituximab to cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP) chemotherapy provides superior survival to CHOP chemotherapy alone [8, 9], and this combination has achieved consensus as the standard treatment especially in patients with advanced-stage DLBCL.

In PG-DLBCL, prospective analyses have been reported mainly in patients with localized disease treated with rituximab plus CHOP (R-CHOP) chemotherapy [10, 11]. However, the role of R-CHOP chemotherapy followed by radiotherapy in localized disease has not yet been evaluated. On the other hand, in advanced disease, there has been no detailed data in patients treated with R-CHOP chemotherapy even retrospective series. Here, we retrospectively analyzed a cohort of 95 patients with localized- and advanced-stage PG-DLBCL receiving R-CHOP chemotherapy with or without radiotherapy.

Methods

Patients

We conducted a retrospective analysis of 95 patients who were newly diagnosed with PG-DLBCL from January 1995 to January 2009 at Nagoya University Hospital and seven associated hospitals. PG-DLBCL was diagnosed if lesions were predominantly in the stomach when the expansion of disease is checked in full body at initial diagnosis [12]. Clinical stage was evaluated according to the Lugano staging system for gastrointestinal non-Hodgkin's lymphoma [13], in which stages I and II1 are categorized as localized disease, and II2, IIE, and IV as advanced disease [13]. All patients received staging investigations, including physical examination, laboratory data analysis, computed tomography (CT) of the chest and abdomen, gallium scintigraphy, or fluorine-18-fluorodeoxyglucose positron emission tomography, bone marrow aspiration/biopsy, and gastrofiberscopy (GF) with biopsy. Evaluation of central nervous system (CNS) involvement was by either or both computed tomography/magnetic resonance imaging and lumber puncture with cerebrospinal fluid analysis where indicated. The following clinical and laboratory data were available at the time of diagnosis: age; sex; performance status (PS); presence of B symptoms, bulky mass, bone marrow involvement, and CNS involvement; serum lactate dehydrogenase (LDH) level; clinical stage; and number of extranodal sites. For this study, International Prognostic Index (IPI) scores were determined, and the patients were categorized into low- (score 0–2) or high-risk groups (score 3–5) [14]. This study was approved by the institutional review board at each participating hospital and complied with all provisions of the Declaration of Helsinki.

Pathological studies

Histological sections were reviewed, and diagnosis was confirmed as DLBCL according to the fourth edition of the World Health Organization Classification of Tumors of Hematopoietic and Lymphoid Tissues [15]. The review was performed by two pathologists (S.N. and T.T.) at the Department of Pathology and Clinical Laboratories, Nagoya University Hospital. Immunohistochemical staining and scoring for CD10, BCL-6, and MUM-1/IRF4 were performed on formalin-fixed paraffin-embedded tissues from patients diagnosed with PG-DLBCL and scored as positive if 30% or more of tumor cells were labeled [16]. The patients were then assigned as germinal center B cell-like (GCB) phenotype or non-GCB phenotype using the algorithm of Hans et al. [16].

Treatment

Analysis was restricted to patients who received CHOP chemotherapy (CHOP or CHOP-like regimen) plus rituximab (R-CHOP) or R-CHOP chemotherapy followed by radiotherapy as initial therapy. Rituximab dosage for all patients was 375 mg/m^2. Therapeutic strategies were determined by the attending physician in each hospital. Regarding localized-stage PG-DLBCL, selection of R-CHOP chemotherapy alone, or R-CHOP chemotherapy followed by radiotherapy was not decided in advance of diagnosis.

Response to treatment

Complete response (CR) was defined as the disappearance of all clinical evidence of disease, negative gastric biopsy, and recovery of all laboratory and radiological abnormalities related to the disease. Partial response (PR) was indicated by a decrease of more than 50% in the sum of the products of the maximum perpendicular diameters of each measurable lesion. Progressive disease (PD) was indicated by at least a 25% increase in the size of any preexisting lesions or by the appearance of any new lesions during or after therapy. Stable disease was neither PR nor PD. Relapse disease (RD) was the appearance of any new lesion in patients who had achieved CR. Overall survival (OS) was defined as the time from initial diagnosis to the date of death from any cause or of last follow-up. PFS was defined as the duration from initial diagnosis to the date of progression, relapse, death from any cause, or last follow-up, whichever occurred first.

Gastrointestinal-specific toxicities

Gastrointestinal-specific toxicities such as gastric hemorrhage, gastric perforation, and gastric obstruction during initial treatment were evaluated. Gastric hemorrhage was defined as symptoms of melena or hematemesis and the presence of hemorrhage confirmed by GF; gastric obstruction as symptoms of vomiting, eating difficulty, and the presence of stenosis confirmed by GF; and gastric perforation as the presence of free air around the stomach in the abdominal cavity on CT.

Statistical analysis

Patient characteristics between treatment groups were compared with Fisher's exact test and median age with the Mann–Whitney U test. OS and PFS were assessed by the Kaplan–Meier method and compared between groups by the log-rank test. The impact of independent prognostic factors on OS was evaluated by univariate and multivariate analyses using a Cox proportional hazards model. Variable factors were as follows: sex; age; performance status; presence of B symptoms, bulky mass, and bone marrow involvement; expression of the GCB phenotype; number of extranodal sites; serum LDH level; addition of rituximab; and addition of radiotherapy. All P values were based on two-sided tests and P values less than 0.05 were considered significant. All statistical analyses were performed using the Statistical Software Package for the Social Sciences (SPSS version 11.0 for Windows; SPSS Inc., Chicago, IL).

Results

Patient characteristics

Patient characteristics are shown in Table 1. Of the 95 patients analyzed in this study, 50 were male and 45 were female with a median age of 68 years (range, 32–86 years). The proportion of GCB phenotype was lower compared with that of non-GCB type (42% and 58%, respectively). Seven variables showed a significant difference between localized- and advanced-stage groups, namely PS, number of extranodal sites, serum LDH level, IPI risk group, bulky mass, and radiotherapy. Frequent extranodal involvements other than the stomach were liver in four patients, spleen duodenum, and bone marrow in three patients and bone in two patients. *Helicobacter pylori* infection was found in 27 of 49 patients (55%) who could be examined for *H. pylori* status in PG-DLBCL. Eleven of 27 patients (41%) with *H. pylori*-positive PG-DLBCL received eradication therapy before or after initial chemotherapy. In 95 patients diagnosed with PG-DLBCL, eight patients (8%) had DLBCL with marginal-zone lymphoma of mucosa-associated lymphoid tissue (MALT) component. *H. pylori* status was recognized in four of six patients (67%) with DLBCL in the presence of MALT component and not examined in two patients. In eight patients of DLBCL with MALT component, all patients were classified into non-GCB phenotype on the immunohistochemical staining.

Treatment

Of the 58 patients with localized disease, 35 patients (60%) received a median of three courses (range, three to four) of R-CHOP chemotherapy followed by radiotherapy, while the remaining 23 (40%) received a median of six courses (range, two to eight) of R-CHOP chemotherapy without radiotherapy. Of the 37 patients with advanced disease, 35 patients (95%) received R-CHOP chemotherapy alone and CHOP (n=35) or CHOP-like regimen (n=2) combined with rituximab. Two patients (5%) received three cycles of R-CHOP chemotherapy combined with radiotherapy.

Table 1 Patient characteristics

Variable	Total (N=95) N (%)	Localized stage (n=58) n (%)	Advanced stage (n=37) n (%)	P value*
Age				
Median age	68	68	67	0.722
Range	32–86	32–84	35–86	
Sex				
Male	50 (52)	30 (51)	20 (54)	0.824
Female	45 (48)	28 (49)	17 (46)	
Performance status				
0–1	89 (94)	57 (98)	31 (84)	0.013
2–4	6 (6)	1 (2)	6 (16)	
Lugano stage				
I	33 (35)	33 (57)	–	
II1	25 (26)	25 (43)	–	
II2	10 (11)	–	10 (27)	
IIE	4 (4)	–	4 (11)	
IV	23 (24)	–	23 (62)	
Extranodal sites				
Fewer than 2 (stomach only)	81 (85)	58 (100)	23 (62)	<0.0001
2 or more	14 (15)	0	14 (38)	
Serum LDH level				
Elevated	29 (31)	9 (15)	20 (54)	0.0002
IPI score				
<3	75 (79)	57 (98)	18 (49)	<0.0001
≥3	20 (21)	1 (2)	19 (51)	
B symptom present	19 (20)	10 (17)	9 (24)	0.438
Bulky mass present	9 (9)	1 (2)	8 (22)	0.002
Bone marrow involvement	3 (3)	0	3 (8)	0.056
Treatment				
Six cycles of R-CHOP	58 (61)	23 (39)	35 (95)	<0.0001
Three to four cycles of R-CHOP +Radiotherapy	37 (39)	35 (61)	2 (5)	
ASCT				
Yes	1 (1)	0	1 (3)	0.389
No	94 (99)	58	36 (97)	
Hans' algorism				
GCB phenotype	40 (42)	22 (37)	18 (49)	0.302
Non-GCB phenotype	45 (58)	36 (63)	19 (51)	

Abbreviations: *LDH* lactate dehydrogenase, *ASCT* autologous stem cell transplantation, *GCB* germinal center B cell-like

*P values are for the comparison of localized- and advanced-stage group

Efficacy

Localized-stage patient

Of the 58 patients with localized disease, 51 patients (88%) and seven patients (12%) achieved CR and PR. No patient developed PD. With a median follow-up for surviving patients of 34.5 months (range, 4.9–89.3 months), 3-year PFS and OS were 93%. With regard to radiotherapy, CR rate in the localized disease was 83% and 91% in six cycles of R-CHOP and in three to four cycles of R-CHOP plus radiotherapy, respectively. 3-Year PFS and OS were 91% and 91% in patients with six cycles of R-CHOP and 92% and 95% in those with three to four cycles of R-CHOP plus radiotherapy (Log-rank test, P=0.595 and P=0.278, respectively; Fig. 1a, b). Twenty-two patients (38%) were classified as the GCB phenotype and 36 (62%) as the non-GCB phenotype. No significant difference in 3-year OS was seen between the GCB and non-GCB phenotypes (92% vs 96%; P=0.886).

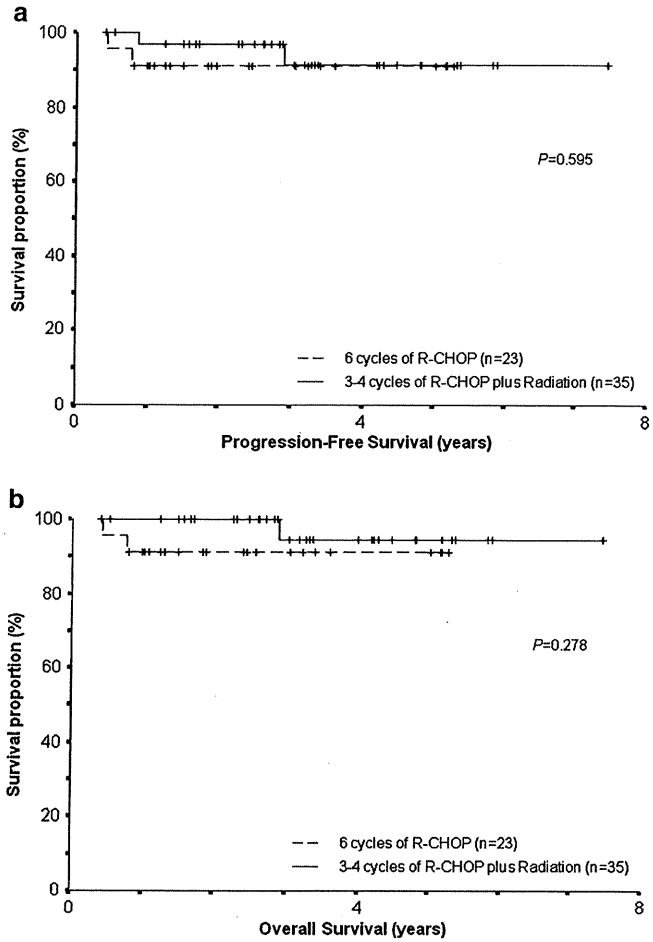

Fig. 1 **a** Progression-free and **b** overall survival of 58 patients receiving six cycles of R-CHOP (n=23) and three to four cycles of R-CHOP plus radiotherapy (n=35) in localized disease

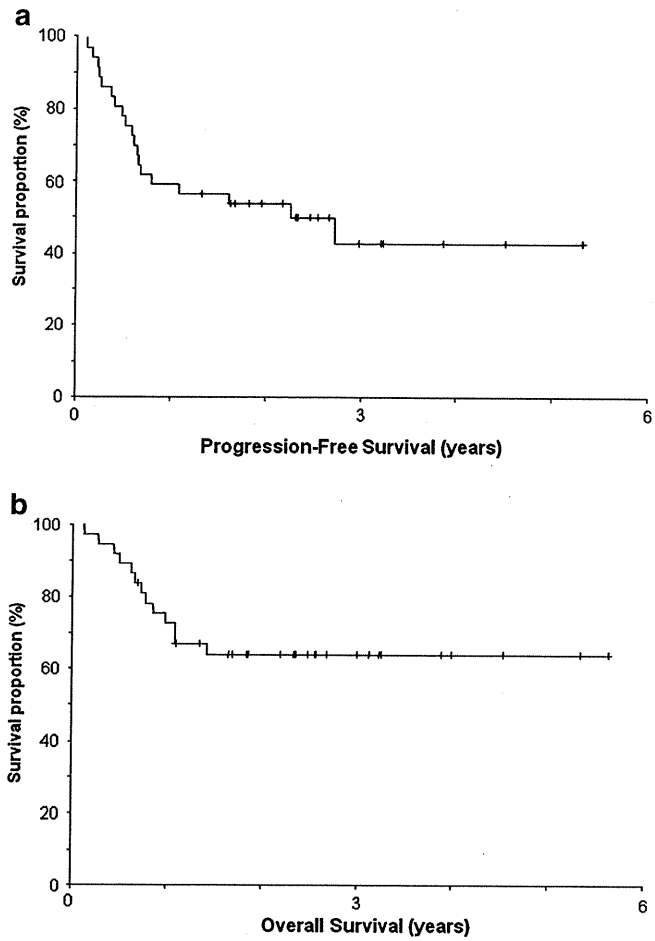

Fig. 2 **a** Progression-free and **b** overall survival of 37 patients receiving R-CHOP chemotherapy with or without radiotherapy in advanced disease

Advanced-stage patient

Of the 37 patients with advanced disease, 29 (78%) and two (5%) achieved CR and PR. Four patients (11%) developed PD. With a median follow-up for the surviving patients of 30.2 months (range, 8.2–67.5 months), 3-year PFS and OS were 43% and 64%, respectively (Fig. 2a, b). Eighteen patients (49%) were classified as the GCB phenotype and 19 (51%) as the non-GCB phenotype. No significant difference in 3-year OS was seen between the GCB and non-GCB phenotypes (58% vs 71%; P=0.303).

Toxicity

Surgical events such as gastric hemorrhage, gastric perforation, and gastric obstruction are shown in Table 2. Gastric perforation was not identified in any patient. Gastric hemorrhage occurred in one patient (1%) in the localized stage and two (5%) in the advanced stage, and gastric obstruction in two patients (3%) in the localized stage and four (5%) in the advanced stage. The frequency of gastric hemorrhage and gastric obstruction between the localized and advanced stage did not significantly differ (P=0.558 and P=0.999, respectively).

Relapsed disease

Localized-stage patient

Among the 51 patients achieving CR after initial treatment, only one patient (2%) developed RD in lymph node with 10.4 months of interval between initial diagnosis and relapse (Table 3).

Advanced-stage patient

Among 29 patients achieving CR, eight patients (28%) developed RD. Sites of relapse were lymph node (n=3), stomach (n=2), CNS (n=2), and duodenum (n=1). Median interval between initial diagnosis and relapse was 7.8 months (range, 4.9–32.9 months). In patients with RD in the CNS or stomach, median interval between initial

Table 2 The frequency of gastric perforation, hemorrhage, and obstruction

Variable	Localized stage (n=58)			Advanced stage (n=37)			
	Six cycles of R-CHOP (n=23)	Three to four cycles of R-CHOP+radiation (n=35)	Total	Six cycles of R-CHOP (n=35)	Three to four cycles of R-CHOP+radiation (n=2)	Total, N (%)	P value
Hemorrhage	0	1	1 (1)	2	0	2 (5)	0.558
Perforation	0	0	0	0	0	0	
Obstruction	1	1	2 (3)	2	0	2 (5)	0.999

diagnosis and relapse was 5.4 and 30.0 months, respectively (Table 3). Of the two patients relapsed in stomach, one was *H. pylori*-positive DLBCL with MALT component and achieved CR with six cycles of R-CHOP chemotherapy. Eradication therapy was not performed before or after chemotherapy. MALT lymphoma occurred in the same lesion of the stomach 27 months later. After eradication therapy, the relapsed lesion disappeared. The other who was *H. pylori*-negative DLBCL relapsed with DLBCL in different lesion of the stomach 32 months later.

Prognostic factors

All patients with localized and advanced disease were analyzed together. In univariate analysis, seven factors were associated with shorter survival, namely poor performance status, involvement of two or more extranodal sites, advanced stage, elevated serum LDH level, presence of bulky mass, presence of B symptoms, and presence of bone marrow involvement. The other three factors, namely sex, age, and expression of the GCB phenotype were not predictive of survival on univariate analysis. In addition, the GCB phenotype was not predictive of survival in both patients with localized and advanced group. Multivariate analysis identified advanced stage (hazard ratio (HR), 4.807; 95% confidence interval (CI), 1.075-21.739; P=0.039) and elevated serum LDH level as independent predictors of survival (HR, 4.901; 95% CI, 1.035–23.255; P=0.045; Table 4).

Discussion

We found that the clinical outcomes in patients with localized-stage PG-DLBCL were extremely favorable in the both groups treated with three cycles of R-CHOP plus radiotherapy and six cycles of R-CHOP, and those tended to be similar. Furthermore, the clinical outcome in patients with advanced-stage PG-DLBCL treated with R-CHOP chemotherapy might be poor. Although retrospective, these findings might be informative in patients with PG-DLBCL in the rituximab era.

In this study, patients with localized-stage PG-DLBCL treated with six cycles of R-CHOP had a CR rate of 83% and 3-year OS of 91%. There have been two reported studies that have prospectively evaluated PG-DLBCL mainly in localized-stage using R-CHOP chemotherapy alone as follows: Wohrer et al. reported a CR rate of 87% (13 of 15 patients) in patients treated with six cycles of R-CHOP [10]. Aviles et al. showed 5-year OS of 95% in 42 patients treated with six cycles of R-CHOP [11]. Although current study was retrospective, our

Table 3 Site of relapse in patients with a CR after initial therapy

Case no.	Age/sex	Stage	Lugano	LDH	IPI score	Extranodal involvement (excluding stomach)	Therapy	Course	Site of relapse	Time to relapse (months)
1	52/F	Localized	I	294	1		R-CHOP+Rad	3	Cervical LN	11.1
2	57/M	Advanced	II2	461	1		R-CHOP	8	CNS	5.8
3	53/M	Advanced	IIE	220	0	Duodenum	R-CHOP	8	Duodenum	8.0
4	71/M	Advanced	IV	398	3		R-CHOP	6	CNS	4.9
5	57/M	Advanced	IV	237	2	Spleen, liver	R-CHOP	7	Mediastinal LN	6.2
6	35/M	Advanced	IV	209	1		R-CHOP	6	Stomach	27.2
7	73/F	Advanced	IV	188	2		R-CHOP	8	Stomach	32.9
8	67/M	Advanced	IV	390	3		R-CHOP	8	Paraorta LN	7.6
9	69/F	Advanced	IV	434	4	Pancreas	R-CHOP	8	Paraorta LN	20.7

CNS central nervous system

Table 4 Univariate and multivariate analysis for OS in patients with PG-DLBCL

Variable	Subgroup	Univariate analysis Hazard ratio [95% CI]	*P* value	Multivariate analysis Hazard ratio [95% CI]	*P* value
Sex	Female vs. male	1.129 [0.420–3.039]	0.885	3.636 [0.952–13.888]	0.058
Age	<60 vs. ≥60	2.096 [0.596–7.352]	0.248	3.194 [0.605–16.949]	0.171
Performance status	0–1 vs. 2–4	5.917 [1.893–18.518]	0.002	2.028 [0.458–8.928]	0.351
Extranodal site	One vs. two or more	3.846 [1.386–10.638]	0.009	1.381 [0.104–7.209]	0.660
Lugano stage	Localized vs. advanced	8.064 [2.298–28.571]	0.001	4.807 [1.075–21.739]	0.039
Serum LDH level	Normal vs. high	6.535 [2.267–18.867]	0.0005	4.901 [1.035–23.255]	0.045
Bulky mass	No vs. yes	3.533 [1.137–10.989]	0.029	1.054 [0.252–4.418]	0.942
B symptom	No vs. yes	3.300 [1.125–8.849]	0.018	2.906 [0.822–10.309]	0.097
Bone marrow involvement	No vs. yes	6.250 [1.385–27.777]	0.017	1.738 [0.224–13.484]	0.596
GCB phenotype	GCB vs. non-GCB	1.293 [0.470–3.558]	0.618	1.769 [0.469–6.666]	0.398

CI confidence interval

result was comparable with previous prospective data in localized-stage PG-DLBCL.

Our analysis of all patients treated with rituximab-containing regimen showed that three to four cycles of R-CHOP plus radiotherapy tended to be similar to six cycles of R-CHOP in terms of PFS and OS. These results suggested that the optimal treatment strategy for localized-stage PG-DLBCL in the rituximab era, in other words, the relative merit of three cycles of R-CHOP followed by involved field radiation versus six cycles of R-CHOP thus remains uncertain. Our results support the use of six cycles of R-CHOP without involved field radiation as an important treatment option for localized-stage PG-DLBCL in the rituximab era.

With regard to advanced-stage PG-DLBCL, our study showed that 3-year OS was 64% with half proportion of high-risk group (IPI score ≥3). However, compared with previous study in patients with DLBCL treated with R-CHOP chemotherapy, 3-year OS was similar to patients with DLBCL in high-risk group [14]. In fact, 7 of 12 patients who developed PD or RD died within 1 year after PD or RD despite the use of salvage therapies, and five of eight patients who developed RD did not achieve CR despite salvage therapies. Considering this poor survival for advanced disease, another therapeutic strategy should be developed. In our case, one patient who received autologous stem cell transplantaion (ASCT) in the initial treatment survived without relapse at the end of the study. ASCT in the initial treatment might be worthy of evaluation as a treatment option for advanced patients especially with elevated LDH level as a poor prognostic factor.

We found two notable remarks in the site of relapse. First, relapse in the stomach was frequent, and *H. pylori* eradication therapy should be performed even if CR was obtained, especially in patients with DLBCL with MALT component. Second, CNS relapse was frequent when time to relapse was short (median, 5.4 months). Given previous findings that early relapse in the CNS within 6 months of initial therapy might have been due to subclinical CNS involvement at the time of diagnosis, however, this finding requires careful interpretation [17]. Of the two patients experiencing CNS relapse in the present study, neither of patients had undergone CNS evaluation at initial diagnosis, and the possibility of subclinical CNS involvement at the time of initial diagnosis could not be excluded.

Massive hemorrhage, gastric obstruction, or gastric perforations in patients with PG-DLBCL are surgical events related to chemotherapy and radiotherapy. In previous studies, the rate of these complications with chemotherapy with or without rituximab was 12% to 25% [18, 19]. In our study, however, the rate of surgical events was 7% with no gastric perforation, suggesting that the frequency of surgical complications was not high in the rituximab era.

Several limitations of our study warrant mention. First, this retrospective study might have been influenced by unrecognized bias. Second, the number of treatment courses was not standardized and thus treatment intensity varied. This variation in our present study, which was also present in previous clinical trials for localized DLBCL [9, 20], might have lead to the underestimation of effects.

In conclusion, we found the clinical outcome in patients with localized-stage PG-DLBCL treated with three cycles of R-CHOP plus radiotherapy tended to be similar to six cycles of R-CHOP with an extremely favorable effect. Furthermore, the clinical outcome in patients with advanced-stage PG-DLBCL might be poor even in the rituximab era. Further prospective analyses are warranted.

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Conflict of interest disclosure The authors declare no competing financial interests.

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ORIGINAL ARTICLE

Cladribine combined with rituximab (R-2-CdA) therapy is an effective salvage therapy in relapsed or refractory indolent B-cell non-Hodgkin lymphoma

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Abstract

Although cladribine has been reported to be an active purine analog against indolent B-cell non-Hodgkin lymphoma (B-NHL), there are few reports of combination use of cladribine and rituximab. This multicenter phase II study evaluated the efficacy and toxicity of cladribine with rituximab (R-2-CdA) therapy in relapsed or refractory indolent B-NHL. Twenty patients with the median age of 58.5 yrs (range, 42–72) were enrolled and received R-2-CdA therapy from April 2005 to July 2007. The median number of prior regimens was 2 (range, 1–3), and fifteen patients (75%) were previously treated with rituximab-containing regimens. Disease histology included follicular lymphoma in 16 patients, MALT lymphoma in two patients, nodal marginal B-cell lymphoma in one patient, and lymphoplasmacytic lymphoma in one patient. The overall response rate (ORR) was 90%, with a complete response rate (CRR) of 70%. Estimated median progression-free survival (PFS) time was 22.4 months (95%CI, 10.9–32.6 months) at a median follow-up time of 27 months (range, 12–43). Two-year PFS and 2-yr overall survival (OS) were 52.6% (95%CI, 31.0–73.2%) and 89.5% (95%CI, 66.1–97.3%), respectively. Grade 3 or grade 4 toxicities were neutropenia in 74% and thrombocytopenia in 11%. R-2-CdA therapy was demonstrated to have a high activity with durable PFS and acceptable toxicity in relapsed or refractory indolent B-NHL mostly pretreated with rituximab-containing therapy. Although a large-scale trial is needed for confirmation, R-2-CdA therapy could be a good salvage therapy option in relapsed or refractory indolent B-NHL.

Key words cladribine; indolent B-NHL; rituximab; salvage therapy

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The indolent B-cell non-Hodgkin lymphoma (B-NHL) is characterized by a protracted natural history. This category consists of follicular lymphoma, extranodal marginal zone lymphoma of mucosa-associated tissue (MALT lymphoma), nodal marginal zone lymphoma, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), and other rare entities. The initial treatment of indolent B-NHL has not been standardized. Thus, several options, including chemotherapy, radiation therapy, and watchful waiting, are used. Almost all B-cell lymphomas express CD 20 antigen on the cell surface. Rituximab, a chimeric anti-CD20 monoclonal antibody, was developed and is

now widely used to treat B-cell lymphoma. A significant improvement in clinical outcomes by the introduction of rituximab in both aggressive (1, 2) and indolent B-NHL was shown by prospective clinical trials (3, 4). Although rituximab-containing chemotherapy showed improved survival in previously untreated advanced-stage follicular lymphoma, follicular lymphoma is not curable as before, and patients usually have several remissions and recurrences during a long clinical course. Because the standard of care have not been established in salvage setting as same as induction setting in first line therapy in indolent B-NHL, development of much more effective salvage regimen is eagerly awaited.

Cladribine (2-chlorodeoxyadenosine, 2-CdA) is a deoxyadenosine purine nucleoside analog that has cytotoxic actions to both resting and proliferating lymphocytes (5, 6). Cladribine is one of the standards of cares for hairy cell leukemia (7–10). Furthermore, cladribine has been shown to be effective against other types of indolent NHL both as a single agent and in combination therapy (11–24). Fludarabine, another well-known purine analog, was used in combination with rituximab in some clinical trials, and favorable clinical outcomes have been reported (25–28). When this study was planned, there was only one approved purine analog, cladribine, in Japan. There are few reports of combination usage of cladribine and rituximab (15, 20). Thus, we conducted a multicenter phase II study to evaluate the efficacy and toxicity of cladribine with rituximab (R-2-CdA) therapy for patients with relapsed or refractory indolent B-NHL.

Patients and methods

Patients

Patients aged 20–74 yrs with measurable, histologically confirmed, relapsed or refractory indolent B-NHL according to the World Health Organization (WHO) classification, version 3 (29), were eligible if they had a life expectancy > 12 wks, an Eastern Cooperative Oncology Group performance status (30) of 0–2, and adequate organ function. A 4-wk wash-out period following prior treatment (12 wks for antibody therapy) was required. Patients were excluded if they had an apparent infection; serious hepatic, renal, cardiac, gastrointestinal, or nervous system disorder; any active malignancy other than lymphoma; or autoimmune hemolytic anemia. Patients treated with cytokines or transfusions within 2 wks prior to registration or with other investigational drugs within 3 months prior to registration were also excluded. Positivity for hepatitis B virus surface antigen, hepatitis C antibody, or human immunodeficiency virus antibody was also excluded. The study was approved by the institutional review board of each participating institution. All patients were required to provide written informed consent. This study was registered in the university hospital medical information network clinical trial registry (UMIN-CTR; number UMIN000001570).

Agents	d1	d2	d3	d4	d5	d15
Cladribine (0.09 mg/kg) (2 h intravenous bolus)	↓	↓	↓	↓	↓	
Rituximab (375 mg/m^2)	↓					↓

Repeated every 28 d for 4 cycles

Figure 1 Treatment protocol of R-2-CdA treatment.

Treatment

Patients received 0.09 mg/kg of cladribine intravenously over 2 h on days 1–5 and 375 mg/m^2 of rituximab intravenously on days 1 and 15, every 4 wks, for a total of four cycles (R-2-CdA, Fig. 1). At the end of each cycle, the toxicity was assessed, and if hematologic and other organ function did not meet the criteria, the next cycle was delayed. If the postponement lasted longer than 21 d, protocol treatment was terminated.

Prophylactic use of trimethoprim/sulfamethoxazole and acyclovir was recommended. Permitted supportive treatment included G-CSF, blood transfusion, and prophylactic use of antibacterial and/or antifungal agents.

Study endpoints, response criteria, and toxicity criteria

This was a single-arm, phase II study. The primary endpoint was the overall response rate (ORR). Secondary endpoints were the complete response rate (CRR), 2-yr progression-free survival (2-yr PFS), and 2-yr overall survival (2-yr OS). The responses of measurable lesions were evaluated by CT scan after the second and fourth cycles of protocol treatment. The response was assessed according to the International Workshop Criteria for non-Hodgkin lymphoma (31). Patients were classified according to best tumor response: complete response (CR), complete response unconfirmed (CRu), partial response (PR), stable disease (SD), or disease progression (PD). The ORR was calculated as the proportion of patients who achieved a CR, CRu, or PR.

Progression-free survival was defined as the time from the enrollment of the protocol treatment to disease progression or death from any cause. OS was measured from the enrollment of the protocol treatment to death or last contact.

Toxicity was graded according to the Common Toxicity Criteria for Adverse Events v3.0 (32). The Follicular Lymphoma International Prognostic Index (FLIPI) (33)

was calculated by summing the number of risk factors (age > 60 yrs, Ann Arbor clinical stage ≥ 3, hemoglobin level < 12 g/dL, number of nodal areas > 4, and lactate dehydrogenase level above normal range). FLIPI was evaluated for each patient at registration.

Statistical analysis and sample size

This study was designed assuming a threshold ORR of 50% to detect an expected ORR of 70%. At the 5% level of significance (one-tailed), 37 patients were required to attain a statistical power of 90%. OS and PFS curves were calculated using the method of Kaplan and Meier. The changes in immunologic variables were evaluated using the Wilcoxon signed-rank test. The date was analyzed statistically using JMP 8 (SAS Institute, Cary, NC, USA).

Results

Patients' characteristics and disposition

A total of 20 patients were enrolled and received R-2-CdA therapy from April 2005 to July 2007. Because the enrollment for this study could not reach the planned sample size (37 patients) during planned enrollment period, this study was stopped on July 2007. The patients' characteristics are shown in Table 1. Their median age was 58.5 (range, 42–72) years. Disease histology included follicular lymphoma in 16 patients, MALT lymphoma in two patients, nodal marginal B-cell lymphoma in one patient, and lymphoplasmacytic lymphoma in one patient; 55% (11 patients) had stage III or stage IV disease. The median number of prior regimens was 2 (range, 1–3). Fifteen patients (75%) were previously treated with rituximab, and two patients had undergone high-dose chemotherapy with autologous stem cell transplantation.

All patients received four cycles of R-2-CdA. The planned treatment schedule of R-2-CdA was 99 d for four cycles, and the median actual treatment duration of four cycles of R-2-CdA in 19 assessable patients was 100 d (range 99–170 d). Administration of cladribine was postponed in three cases because of hematologic toxicity according to the protocol definition. All except for one case, in whom three doses of rituximab were omitted because of infection (grade 1), received the planned doses of cladribine and rituximab without dose reduction.

Efficacy

The ORR was 90% (18/20) with the CRR of 75% (15/20) (Table 2). There was no relationship between response status (CR or not) and FLIPI at registration (low, intermediate, or poor risk) (P = 0.38, Mann–Whitney U test). The efficacy in patients previously treated with rituximab-containing therapy (rituximab exposure group) was similar to that in patients without previous rituximab exposure (rituximab naïve group) (P = 0.77, Fisher's exact test) (Table 2). The estimated median time PFS was 22.4 months (95% CI, 10.9–32.6 months) at a median follow-up time of 27 months (range, 12–43). The 2-yr PFS and 2-yr OS were 52.6% (95%CI, 31.0–73.2%) and 89.5% (95%CI, 66.1–97.3%), respectively (Fig. 2). There was no significant difference (P = 0.186) in 2-yr PFS between rituximab exposure group (42.9%) and rituximab naïve group (80.0%). FLIPI risk group did not affect PFS (data not shown). The majority of patients had follicular lymphoma (16/20), so we also analyzed the 2-yr PFS and 2-yr OS in the follicular lymphoma subset. The 2-yr PFS and 2-yr OS in follicular lymphoma were 53.3% (95%CI, 29.2–76.0%) and 86.7% (95%CI, 59.3–96.7%), respectively (Fig. 2, dotted line).

Table 1 Patient characteristics at registration (N = 20)

Characteristics	No. (%)
Age (yrs)	
Median (58.5)	
Range (42–72)	
Gender	
Male	13 (65)
Histology	
Follicular lymphoma	16 (80)
MALT lymphoma	2 (10)
Nodal marginal lymphoma	1 (5)
Lymphoplasmacytic lymphoma	1 (5)
Clinical stage (Ann Arbor)	
IA	4 (20)
IIA	4 (20)
IIB	1 (5)
IIIA	6 (30)
IVA	5 (25)
Bone marrow involvement	
Positive	5 (25)
FLIPI	
Low risk	13 (65)
Intermediate risk	4 (20)
High risk	3 (15)
Previous chemotherapy	
Median (2)	
Range (1–3)	
1 regimen	10 (50)
≥ 2 regimens	10 (50
Previous anthracyclin	15 (75)
Previous high dose chemotherapy	2 (10)
Previous rituximab	15 (75)

FLIPI, follicular lymphoma international prognostic index.

Toxicity

The toxicity profile of the 19 assessable patients is shown in Table 3. Overall, R-2-CdA was well tolerated. Major

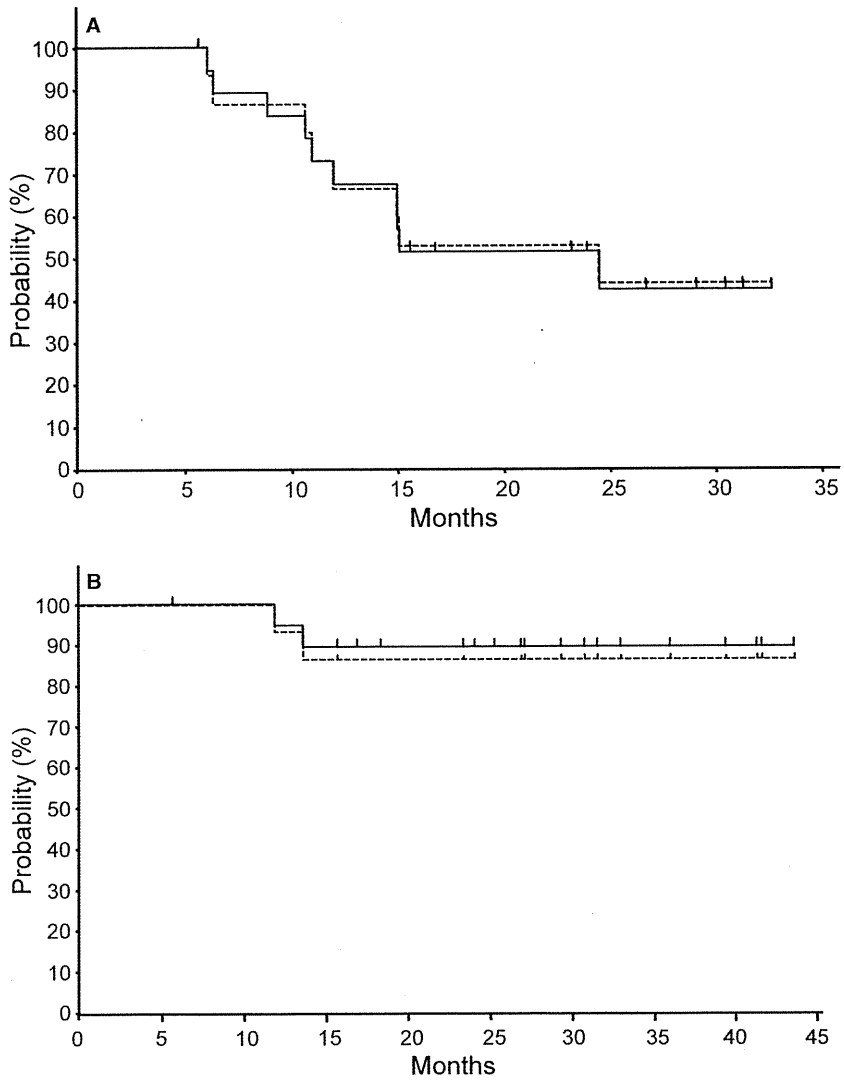

Figure 2 Progression-free survival (PFS) (A) and overall survival (OS) (B) of all 20 patients (solid line) and 16 patients with follicular lymphoma (dotted line). Marks indicate censored observation. 2-yr PFS is 52.6% (95% CI 31.0–73.2%) for all patients and 53.3% for patients with follicular lymphoma (95% CI 29.2–76.0%), and 2-yr OS is 89.5% (95% CI 66.1–97.3%) for all patients and 86.7% (95% CI 59.3–96.7%) for patients with follicular lymphoma.

Table 2 Response rate

Response	No.	%
All assessable patients (N = 20)		
OR	18	90
CR	15	75
PR	3	15
SD	1	5
PD	1	5
Patients previously treated with rituximab (N = 15)		
OR	13	87
CR	11	73
PR	2	13
SD	1	7
PD	1	7

OR, overall response; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

Table 3 Toxicity during this protocol (N = 19)

	Grade (CTCAE v3.0)			
Toxicity	1	2	3	4
Neutropeina	0	2	13	1
Thrombocytopenia	8	2	1	1
Anemia	3	0	2	1
Infection	1	0	0	0
Liver dysfunction	10	2	0	0
Renal dysfunction	3	0	0	0
Others	2	0	0	0

severe toxicity was hematologic. Grade 3 or 4 toxicities were neutropenia in 14 patients (73.7%), thrombocytopenia in two patients who never needed platelet transfusion, and anemia in two patients. Among these patients, prolonged grade 3 or 4 neutropenia and thrombocytopenia lasting for more than 100 d were observed in two patients each. There was no febrile neutropenia.

Non-hematologic toxicity as generally mild and grade 3 or greater non-hematologic toxicity was not observed. The frequency of liver dysfunction was relatively high

Table 4 The changes in immunologic variables ($N = 13$)

	Pretreatment		Post-treatment		Wilcoxon signed-rank test
	Median	Range	Median	Range	
IgG (mg/dL)	972	469–1991	973	547–1639	0.849
IgA (mg/dL)	163	45–859	166	47–680	0.791
IgM (mg/dL)	73	23–2440	56	13–1065	0.013
CD4 (cells/μL)	294	154–1425	102	30–292	0.0002
CD8 (cells/μL)	376	125–1776	283	11–540	0.0942

(63.2%), but almost were grade 1. All non-hematologic toxicities were fully recovered.

Immunologic variables were also assessed. The changes in serum IgG, IgM, and IgA levels, and $CD4^+$ T-cell and CD8 + T-cell counts between at registration and completion of the protocol could be evaluated in 13 patients (Table 4). CD 4 counts decreased significantly after the protocol treatment ($P = 0.0002$).

Discussion

In this study, R-2-CdA therapy was demonstrated to have high activity with durable PFS and acceptable toxicity in relapsed indolent B-NHL. The enrollment for this study could not reach the planned sample size. This final poor accrual rate (54%, 20/37) might be because of the emergence of other new agents such as ibritumomab tiuxetan as a radioimmunotherapy (34) and oral fludarabine (35). It is noteworthy that the high ORR of 90% was shown, although no statistical conclusion can be drawn because of small enrolled number of patients. This high efficacy was also seen in patients previously treated with rituximab-containing therapy.

Cladribine is an active purine analog useful against indolent lymphoid malignancies, and it has been established as standard treatment for hairy cell leukemia (7–10). In Western countries, fludarabine, another purine analog, has been widely used for the treatment of indolent B-NHL (25–27). The basic structures of both agents are very similar, and the difference in the anti-lymphoma effect of these drugs has not been elucidated. Tondini *et al.* (12) conducted a randomized phase II study of fludarabine and cladribine for relapsed or refractory low-grade NHL, and both agents were comparably effective (ORR: 68% and 72%, respectively). Recently, a randomized phase III study comparing the efficacy of cladribine and fludarabine, each combined with cyclophosphamide, in untreated CLL was reported (24). Both combinations were shown to have equal effect and safety in CLL. Karlsson *et al.* (36) compared cladribine, fludarabine, and chlorambucil for CLL, and reported the longer response duration in cladribine group than other agent groups. All these studies suggested that purine analogs were highly effective in indolent B-NHL including CLL.

The combinations of purine analogs with rituximab for relapsed indolent B-NHL were widely examined. Fludarabine was used in combination with rituximab in several clinical trials, and favorable clinical outcomes have been reported (25–28). A few studies of the combination therapy of cladribine and rituximab were reported, and showed high efficacy (15, 20). Also in this study, the favorable ORR and PFS comparable with those in phase II studies of intravenous or oral fludarabine in combination with rituximab were observed (25, 28). The single-agent phase II study of six cycles of cladribine (0.09 mg/kg for seven consecutive days) showed ORR of 58.1% and 2 yrs PFS of 30.3% (18). In this study, total four cycles of cladribine (0.09 mg/kg for five consecutive days) combined with eight doses of rituximab presented the favorable efficacy (ORR 90%; 2 yrs PFS 52.6%). This might indicate that the rituximab combination improved the efficacy even if the reduced cycles and doses of cladribine administration. Recently, subcutaneous injection of cladribine combined with rituximab was shown to be highly effective for Waldenstrom macroglobulinemia (15). Such administration is easy and convenient especially for outpatient clinic. The combinations of purine analogs and rituximab now are getting to be a promising modality for the relapsed and refractory indolent B-NHL.

The hematologic toxicities were major adverse events of cladribine. Severe and prolonged cytopenia has been reported when it was administered as 0.09 mg/kg/d with a schedule of 7-d continuous infusion or 1.4 mg/kg/d with a schedule of 5-d, 2-h infusion (12, 18). Furthermore, 5-d infusion of cladribine (0.1 mg/kg/d) as a subcutaneous bolus administration was reported to maintain the equivalent antitumor activity to 7-d continuous infusion (0.1 mg/kg/d) and show safer toxicity profiles than continuous infusion in indolent NHL (21). According to pharmacokinetics study of cladribine, there was no difference in area under the concentration vs. time curve in tumor cells between 24-h continuous infusion and intermittent 2-h infusion (37). For these reasons, four cycles of 2-h infusion regimen of 0.09 mg/kg/d (approval daily dose in Japan) for 5 d was adopted for combination with rituximab in this study. Grade 3 or greater neutropenia developed in 17 of 19 patients, but there were no episodes of febrile neutropenia. Prolonged cytopenias were developed in 2 out of 19 patients (11%), and the frequency seemed to be lower than that of six cycles of 7-d infusional cladribine monotherapy (16.8%) (18), but was not statistically different. All 20 patients received the planned dose and course of cladribine, and this tolerability might be because of this dose modification. Despite this modification, this protocol showed high efficiency and well-tolerable toxicity.

Immunologic suppression by the combination of a purine analog with rituximab has been suggested, and marked decreases in CD3 + T and CD19 + B cells have been reported (25, 38). In this study, the serum immunoglobulin (IgG, IgA, and IgM) and the $CD4^+$ T-cell and CD8 + T-cell counts were measured before the start of treatment and after the completion of treatment in 13 patients. As previously reported in monotherapy study of 2-CdA (18), significant decreases in $CD4^+$ T-cell counts were also observed in this R-2-CdA therapy. However, the level of serum immunoglobulin was not affected (IgM decreases might be because of improvement in lymphoma-associated paraproteinemia by this therapy).

In conclusion, this study suggested cladribine and rituximab combination therapy seems to be highly effective for relapsed and refractory indolent B-NHL with acceptable toxicity. Although a further large-scale trial is needed, R-2-CdA therapy could be a good salvage therapy option in relapsed indolent B-NHL.

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